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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Monty Krieger, Susan L. Acton, and Alan M. Pearson

Serial No.: 08/265,428 Group Art Unit: 1812

Filed: June 23, 1994 Examiner: J. Ulm

For: CLASS BI AND CI SCAVENGER RECEPTORS

Assistant Commissioner
for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.131

Sir:

We, Monty Krieger and Susan L. Acton, hereby declare that:

1. We are the co-inventors of the claimed subject matter in the above-identified patent application.

2. We are familiar with the publication by Calvo, et al., *J. Biol. Chem.*, 268(25): 18929-18935, (September 5, 1993). Calvo, et al. isolated a human gene encoding a protein of unknown function based on its homology to CD36 and LIMPII. After we determined the structure and ~~function~~ a functional activity (S.A. 4/24/96) MK(4/30/96) of the hamster class B1 scavenger receptor protein, described and claimed in the above-identified patent application, it was apparent to us that the gene isolated by Calvo, et al. encodes the human homologue of the hamster class B1 scavenger receptor protein.

3. As demonstrated by the enclosed copies of pages from the laboratory notebook of Susan Acton, Attachment A, from which the dates have been deleted, we had conceived and reduced to practice in our laboratory at the Massachusetts Institute of Technology, Cambridge, MA, an isolated scavenger receptor protein type BI, as claimed, prior to cDNA encoding (id (i 4/28/96) CK (4/30/96 September 5, 1993. Specifically, we isolated a cDNA clone, inserted the clone into a plasmid which we transfected into COS cells and then screened the transfected COS cells for endocytosis of fluorescently labeled acetylated low density lipoprotein (AcLDL). COS cells do not endocytose AcLDL. Therefore, the transfected cells exhibiting endocytosis of AcLDL expressed the claimed receptor encoded by the isolated clone. Competitive binding assays were also performed prior to September 5, 1993, to determine inhibition of uptake of AcLDL by other common ligands for macrophage scavenger receptors, poly I and m-BSA. Specifically, Attachment A shows:

i. The creation of expression library pools (pages 1-3);

ii. PCR and minipreps of colonies to check cDNA sizes (pages 4-7);

iii. Transfections of COS M6 cells with cDNA pools; screening uptake of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanineperchlorate-labeled acetylated low density lipoprotein (DiI-AcLDL) by the transfected cells but not the non-transfected control; and, identification of positive expression isolates which exhibited binding to AcLDL (pages 8-9);

iv. Subcloning of positive isolates, re-screening with DiI-AcLDL (pages 10-13);

v. Competitive inhibition studies of subclones with poly I and m-BSA (page 12); and,

vi. Further subcloning of clones from cells exhibiting uptake of DiI-AcLDL and having the competitive binding properties of CD36 (pages 14-19).

4. As demonstrated by the enclosed copies of pages from the laboratory notebook of Susan Acton (dates have been removed), Attachment B, from which the dates have been deleted, we had obtained partial nucleotide sequence of the isolated clone which we used to conduct computerized sequence comparisons in six different data banks: PDB, SwissProt, PIR, SPupdate, GenPept, and GPupdate, prior to September 5, 1993. The search indicated that the sequences for LimpII and CD36 were among the highest scoring segment pairs, and that CLA-1 was not in the databases at that time.

5. The entire nucleotide sequence was obtained and compared to the nucleotide sequence of CLA-1 after September 5, 1993. There was 81% identity of amino acid sequence between our scavenger receptor protein and CLA-1. and 80% nucleotide sequence identity. (JL 4/24/96)

6. We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon. (MK 4/30/96)

Date: 4/25/96 Monty Krieger

Date: 4/24/96 Susan L. Acton